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Date of Submission: JANUARY 24, 2007

Selena Whitaker-Paquet

Attorney Docket No.: 9900.1005
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re **Janet A. WARRINGTON, Zhaomei ZHANG, Bertha CHEN, Yan WEN** and **Mary Lake POLAN**

Group Art Unit: 1634

Application No. : 10/684,539

Filed : October 14, 2003

For : **METHODS OF DIAGNOSING AND TREATING STRESS URINARY INCONTINENCE**

Examiner : Sarae Bausch

DECLARATION OF DR. BERTHA CHEN

MAIL STOP: AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

The undersigned, Dr. Bertha Chen, hereby declares:

1. I am an associate professor in the School of Medicine at Stanford University, the assignee of the subject patent application, and an inventor of the claimed subject matter. The following studies were carried out under my supervision.

2. Expression of levels of alpha-1 antitrypsin in vaginal wall tissue samples taken premenopausal women with stress urinary incontinence/pelvic organ prolapse (SUI/POP) were determined by Western blot analysis and QC-PCR analysis and compared with expression levels in tissues obtained from unaffected, control, patients. The results are shown in Figs. 1 and 2 submitted herewith as Exhibits A and B, respectively.

Fig. 1A shows Western blot analysis of alpha-1 antitrypsin in vaginal wall tissue. Lane 1-5 (S1-S5), protein from SUI/POP patients; lane 6-10 (C1-C5), from control patients. The same blot was reprobed with mouse anti-human TGFβ3 monoclonal antibody to confirm that equal amounts of protein were loaded in each lane. Plasma alpha-1 antitrypsin was used as positive control. Fig. 1B shows that protein levels of alpha-1 antitrypsin in SUI/POP patients (mean ± SE, 0.42± 0.078) were significantly lower ($p<0.003$) than those in the control group (mean ± SE, 1.58± 0.27).

Fig. 2A shows QC-PCR analysis of alpha-1 antitrypsin mRNA from vaginal wall tissues in both SUI/POP and control patients. Lane 1-11 (top), PCR products from SUI patients; lane 1-9 (bottom), PCR products from control patients. Fig. 2B shows alpha-1 antitrypsin mRNA level in SUI patients ($N=11$, mean ± SE, 26.6± 7.5) compared to control patients ($N=9$, mean ± SE, 117.2± 37.4). Eight out of nine case-control pairs showed lower alpha-1 antitrypsin mRNA levels compared to controls ($p<0.018$).

3. These results indicate that reduced expression of alpha-1 antitrypsin in vaginal tissues is predictive of the presence of stress urinary incontinence (SUI) and may thus be employed in the diagnosis of SUI or a predisposition to SUI in a patient.

4. The undersigned further declares that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 35 of the United States Code.

Bertha Chen, M.D.

1/16/07

Date